Published in final edited form as:
*Neuroimage*. 2011 September 1; 58(1): 286–292. doi:10.1016/j.neuroimage.2011.05.068.

# Longitudinal diffusion tensor imaging and perfusion MRI investigation in a macaque model of neuro-AIDS: A preliminary study

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## Abstract

The Simian immunodeficiency virus (SIV) infected macaque model exhibits neuropathological symptoms similar to those of $HIV^+$ patients, and is ideal for studying cognitive impairment and neuropathological sequelae of disease in repeated measurements. The aim of this study is to use Diffusion Tensor Imaging (DTI) and perfusion MRI to longitudinally access the disease development in SIV-infected monkeys under controlled conditions and to cross-validate our finding with MRI studies in $HIV^+$ patients. Three adult male pig-tailed macaques (Macaca Nemestrina) were inoculated with the SIVsmmFGb virus. Blood was collected for enumeration of CD4+ and CD8+ T cells. Serial time-sensitive high-resolution $T_2$- weighted structural images, Cerebral Blood Flow (CBF) maps measured with the Continuous Arterial Spin Labeling (CASL) technique, and DTI images were obtained. Animals were sacrificed after 24 weeks. Cognitive behavioral tests were also carried out at each time point. Longitudinal changes in brain volume, CBF, and DTI in selected regions were analyzed statistically. In this study, CD4+ T cell counts were found declined significantly after SIV infection in all macaques. No significant neurological behavior and brain volume changes were observed following virus inoculation. The CBF was found reduced in the caudate, inferior parietal cortex, and the prefrontal cortex. Fractional Anisotropy (FA) values in the whole brain and several Regions of Interest (ROIs) decreased significantly. These longitudinal changes in CBF and FA are correlated with CD4+ T cell depletion and/or CD4:CD8 ratio. The MRI findings from this pilot study agree with previous results in $HIV^+$ patients.



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### Keywords

HIV/AIDS; SIV; Neuro-AIDS; Cerebral blood flow (CBF); diffusion tensor imaging (DTI); non-human primate

## Introduction

MRI is a non-invasive technique which has been used to detect brain abnormalities in $HIV^+$ patients. Many modalities, such as anatomical $T_1$- or $T_2$- weighted structural images, *in vivo* Magnetic Resonance Spectroscopy (MRS), functional MRI, diffusion tension imaging (DTI), and perfusion MRI have been applied in clinical and preclinical studies. Some changes in brain anatomical structure, cerebral metabolism, function, physiology, white matter integrity derived from these modalities have been proposed as potential markers of the development and progression of the disease (Arendt, 1995; Bakshi, 2004; Descamps et al., 2008; Thurnher and Donovan Post, 2008; Tucker et al., 2004). Among them, DTI and perfusion MRI may be most sensitive to HIV development in the brain. In DTI studies of HIV/AIDS patients, it is found that the fractional anisotropy (FA) decreased significantly in the splenium, genu, internal capsule, and whole brain and that this measure correlated well with the severity of the disease. These measures therefore may be particularly strong candidates as surrogates for neurological changes in $HIV^+$ patients (Chen et al., 2009; Filippi et al., 2001; Gongvatana et al., 2009; Muller-Oehring et al., 2010; Pfefferbaum et al., 2007; Pfefferbaum et al., 2009; Pomara et al., 2001; Ragin et al., 2005; Thurnher et al., 2005; Wu et al., 2006). Also, abnormal cerebral blood flow (CBF) has been observed and proposed as a biomarker of disease state in HIV infected patients (Ances et al., 2009; Chang et al., 2000; Ernst et al., 2000; Maini et al., 1990).

In clinical studies, however, it is usually impossible to control for route of infection, viral strain, severity of disease, or time post infection. These uncertainties complicate evaluation of the efficacy of MRI as an index of disease stage. An appropriate animal model, in which controlled and repeated measurements are possible, is therefore a better setting in which to evaluate MRI-based measures as biomarkers of disease progression.

Disease manifestations and progression in Non-human primates (NHPs) infected with Simian immunodeficiency virus (SIV) parallel most aspects of $HIV^+$ patients. Thus, the SIV infected NHP model is of great importance in preclinical AIDS research (Burudi and Fox, 2001; Gold et al., 1998; Murray et al., 1992; Novembre et al., 1994; Williams et al., 2008). It is also an ideal model for the longitudinal evaluation of neuro-imaging markers under controlled conditions, especially with the modern high field MRI techniques, which was the aim of the present study. Specifically, we sought to measure longitudinal and quantitative changes of CBF, FA, mean diffusivity (MD), and brain volume in pig-tailed macaques before and after infection with SIV under controlled condition. Peripheral indices, including CD4+ and CD8+ T-cell counts, as well as several measures of cognition, were also obtained from the monkeys and compared with the MRI findings.

## Materials and methods

### Subjects

Three, 4-year-old male pig-tailed macaques (*Macaca nemestrina*) were infected with 100 $TCID_{50}$ of the neuropathogenic SIVsmmFGb isolate (Novembre et al., 1998; O'Neil et al., 2004). Animals underwent health screening and were determined to be in good health before assignment to the project.

### CD4+ and CD8+ T-cell monitoring

Blood samples were collected from anesthetized animals, for quantitation of CD4+ and CD8+ T-cell subsets, one day before each MRI scan. Absolute numbers of CD4+ and CD8+ T cells were enumerated by flow cytometry as previously described (Novembre et al., 1998; O'Neil et al., 2004).

### Cognitive behavioral tests

The computer-based cognitive behavioral tests were designed to characterize the nature of cognitive function in monkeys (Herndon et al., 1997). The battery included the following tests of cognitive behavior: cued and uncued attention (Gold et al., 1998; Grant et al., 1995), Delayed Non-Matching-to-Sample (DNMS) (including both acquisition and memory performance with delays) (Comparet et al., 1992; Eacott et al., 1994), Delayed Recognition Span- spatial condition (DRST-spatial), and Spatial Reversal (SR) (Martin et al., 1995; Sahakian et al., 1995). All monkeys were evaluated with this battery three times prior to inoculation, and at post-inoculation weeks of 2, 4, 8, 12, 16, 20, and 24 except initial training.

*Analysis of variance* (ANOVA) was used to analyze the performance of behavioral tests with SPSS 17.0, P-values less than 0.05 were considered statistically significant.

### MRI measurements

MRI scans were performed in a Siemens 3T Trio whole body scanner (Siemens Medical, PA, USA) with the Siemens CP extremity volume coil. Animals were immobilized with a custom-made head holder and placed in the sphinx position. Anesthesia was maintained with 1–1.5% isoflurane mixed with O2. Et-$CO_2$, inhaled $CO_2$, $O_2$ saturation, blood pressure, heart rate, respiration rate, and body temperature were monitored continuously. $T_2$-weighted images were acquired with fast spin-echo sequence with the parameters: TR = 3000 ms, TE = 126 ms, FOV = 96 mm × 96 mm, data matrix =128 × 128, slice thickness = 2 mm. The amplitude-modulated CASL technique was used for CBF data acquisition (Wang et al.). The CASL sequence parameters were: TR = 3840 ms, TE = 19 ms, FOV = 96 mm × 96 mm, data matrix = 64 × 64, slice thickness = 2.0 mm, post-labeling delay = 0.8 s, Labeling duration= 2 s, label offset = 50 mm. 16 slices were acquired. Each scan acquired 40 pairs of images and was repeated 3 times. A segmented double-spin echo EPI sequence was used for DTI data acquisition. The parameters were: TR= 4800 ms, TE = 89 ms, FOV= 96 mm × 96 mm, data matrix = 128 × 128, 2 shots, b-values = 0, 1000 s/$cm^2$, 60 gradient directions, 32 slices, and slice thickness = 1.5 mm (voxel size=1.5 × 1.5 × 1.5 $mm^3$). The DTI scan was repeated three times. $T_2$-weighted images, DTI, and resting CBF measurements were acquired in one setting and obtained at the baseline (three times before virus inoculation) and in the weeks of 2, 4, 8, 12, 16, 20 and 24 post-inoculation.

### Data analysis

**Brain atrophy index estimation—**$T_2$ weighted images, taken in the axial plane (Fig 1) were used to calculate several ratios as indices of possible cerebral atrophy. Specifically, the ventricle-brain ratio (VBR) was used to estimate the overall cerebral atrophy, the bicaudate ratio (BCR) to measure caudate region atrophy, and the bifrontal ratio (BFR) to measure frontal region atrophy (Hestad et al., 1993). The VBR was defined as the ratio of the area of lateral ventricles over that of the whole brain in the slice where the brain perimeter is maximized (Fig. 1a). The BCR was the ratio of the minimum inter-caudate distance over the corresponding whole brain width in the same slice (Fig. 1b). The BFR was the ratio of the distance between the lateral tips of the frontal horns over the corresponding whole brain width in the same slice (Fig. 1c). The BCR and BFR were measured in the slice where the

frontal horns were clearly identified while the septum was thinnest. Linear and area measurements were determined using the ImageJ software.

**CBF calculation—**The CBF maps were calculated with the formula (Wang et al., 2005):

$$CBF=\frac{\lambda*\Delta M*R_{1a}}{2a*M_{con}*(\exp(-PLD*R_{1a})-\exp(-(LD+PLD)*R_{1a}))}$$

Where, λ (Brain/blood partition coefficient) = 0.9 (Herscovitch and Raichle, 1985), $R_{1a}$ (relaxizaton time of arterial blood at 3T) = 1.66 sec (Lu et al., 2004). α (labeling efficiency) = 0.6 (Wang et al., 2005), ΔM image intensity difference between control and labeling images, $M_{con}$: image intensity of control images; PLD: post labeling delay; LD: labeling duration.

The results were generated with the custom-made MATLAB scripts. In addition, the CBF values in different slices were calibrated with varying post-labeling delays in consideration of different acquisition time for each slice. The CBF values of each animal were normalized to its baseline value. The caudate, frontal cortex and the inferior medial parietal cortex were selected for ROI analysis (Ances et al., 2006; Chang et al., 2000). Each ROI was selected based upon the correspondent $T_2$-weighted images, and its size was adapted to the representative area of each structure with the Stimulate software (Strupp, 1996) (Fig 3a). The last CBF data set (in the 24$^{th}$ week) was acquired incompletely and excluded during data analysis.

**MD and FA calculation—**In order to reduce the motion artifacts and variation during ROI selection on each time point, SPM (www.fil.ion.ucl.ac.uk/spm) was employed to co-register all DTI images of each animal to the corresponding baseline ones. Each ROI was selected based upon the corresponding $T_2$-weighted images. Cerebrospinal fluid (CSF) was excluded manually during whole brain analyses of FA and MD (Ragin et al., 2004). Splenium and genu of corpus callosum (CC) and frontal white matter (WM) were selected for ROI analysis (Pomara et al., 2001; Thurnher et al., 2005; Wu et al., 2006). In order to eliminate the discrepancy between subjects, the FA and MD values of each animal were normalized to its own baseline values. SPSS was used for statistical analyses.

ANOVA for repeated measures was performed to access the differences across all time points; Student's *t-test* was applied to compare the averaged FA, MD, and CBF pre- and post-inoculation. P-values less than 0.05 were considered statistically significant in repeated ANOVA, *t-test* and correlation analysis. Correlations with the blood T-cell counts were carried out using simple regression analysis.

Baseline values for these analyses were derived from the average of the three scans on each animal before the inoculation. All procedures and protocols were in compliance with the Institutional Animal Care and Use Committee (IACUC) of Emory University.

## Results

### Cognitive tests

Monkeys learned and reliably performed the tests of the cognitive battery, but it was found pre-inoculation performance was not different from post-inoculation behavior on any of the tests.

### CD4+ and CD8+ counts in plasma

Longitudinal changes in CD4+ and CD8+ T-cell counts are illustrated in Fig. 2. CD4+ counts declined progressively as expected, differing significantly from the baseline at weeks 4, 12, 16, 20 and 24 after inoculation in comparison with the baseline. CD8+ counts were significantly decreased immediately after inoculation, and decreased progressively thereafter, with the exception of a temporary increase in the $8^{th}$ week.

### Brain atrophy index measurement

No significant changes were observed in all the measures of VBR, BCR and BFR after inoculation. However, BFR changes are significantly negatively correlated with CD4 counts (Table 1).

### CBF measurement

CBF in the selected ROIs (Fig 3a, caudate, prefrontal cortex, and inferior medial parietal cortex) decreased after the SIV inoculation (Fig 3b). In comparison with the baseline CBF, the CBF reduction was statistically significant in both caudate ($4^{th}$, $8^{th}$ and $16^{th}$ week after viral inoculation), prefrontal cortex ($8^{th}$ week) and inferior medial parietal lobe ($8^{th}$, $20^{th}$ week) (Fig 3c). The CBF changes in the prefrontal cortex generally correlated with the changes of the CD4+ counts, but are not synchronized with the CD4+ counts and lagged behind slightly. The CBF changes of the selected ROIs significantly correlated with CD4+ counts and CD4/CD8 ratio (except prefrontal cortex) (Table 2).

### FA and MD measurement

The whole brain FA and MD before and after inoculation were analyzed with *t-test* and repeated ANOVA for longitudinal comparison (Fig 4). The whole brain FA was significantly reduced after inoculation (Fig. 4a). The whole brain MD showed an increasing tendency after inoculation but did not reach statistical significance (Fig. 4a). Longitudinal analysis revealed that the whole brain FA was reduced significantly in the $2^{nd}$ week and $8^{th}$ week post inoculation in comparison with the baseline value (Fig. 4b). The whole brain MD appeared increased in the $4^{th}$ week post inoculation, but this difference was not significant (Fig. 4b).

The mean FA (averaged across time) in genu of corpus callosum was found significantly reduced after the inoculation, while the one in splenium showed a non-significant decline. Also, there were no significant FA changes observed in the frontal WM (Fig 5b). In the longitudinal analysis, significant declines in the FA of genu were observed 8 and 12 weeks after inoculation (Fig. 5c). The mean MD values in these ROIs showed a trend toward an increase following inoculation (Fig. 5d), but this change was not significant.

The cross-correlations of FA and MD in the whole brain and ROIs with the CD4 counts and CD4:CD8 ratios are shown in Table 3. FA values in the whole brain and splenium correlated significantly with CD4+ cell counts. The FA values in genu and frontal WM correlated significantly with CD4:CD8 ratios.

## Discussion

CD4+ depletion and CD4/CD8 ratio are routinely used to evaluate and track the progression of HIV infection in clinic (Lentz et al., 2009). The progression and development of the SIV infection in the three subjects were illustrated by the longitudinal changes of CD4 and CD8+ T-cell counts (Fig. 2). Significant changes were observed before and after the viral inoculation and at weeks of 4, 12, 16, 20 and 24 after inoculation in comparison with the baseline.

Cognitive behavioral tests are very important in the SIV model because HIV will result in impaired cognitive function (Grassi et al., 1999; Hall et al., 1996; Letendre et al., 2010; Murray et al., 1992) and HIV-associated neurocognitive disorders (HAND) remain highly prevalent in $HIV^+$ patients (Gandhi et al., 2011; Gorman et al., 2009; Woods et al., 2009). However, no significantly abnormal behavior was observed from the neurological examinations of these SIV-infected monkeys. The absence of changes in cognitive behavior performances may indicate that the SIV-infected monkeys were still in the asymptomatic stage of disease development.

### CBF in caudate and inferior parietal cortex

Significant reduction of CBF after inoculation was observed in both caudate and parietal lobe. The result is consistent with previous studies in $HIV^+$ patients (Ances et al., 2006; Ances et al., 2009; Chang et al., 2000; Maini et al., 1990). The CBF in prefrontal cortex showed evident reduction but did not reach statistical significance due to high levels of variability in this measure.

In the longitudinal analysis, a significant decrease of CBF in caudate and parietal lobe was observed in the $4^{th}$ and $8^{th}$ week post inoculation respectively (Fig 3). CBF in prefrontal cortex was observed decreasing sharply right after inoculation but began to increase slightly thereafter. These changes in acute or early stage post inoculation were also showed in other regions in previous neuroimaging studies of $HIV^+$ subjects (Ances et al., 2009). The significant correlations between the longitudinal CBF changes and CD4+ T-cell counts and CD4/CD8 ratio in caudate and parietal regions suggest that CBF is sensitive to the progression of the disease and may be a potential imaging marker.

Reduced CBF in caudate, prefrontal cortex, parietal lobe, etc, has been found to be correlated with the CD4+ counts or severity of dementia in previous studies about HIV-associated brain injury (Ances et al., 2006; Ances et al., 2009; Chang et al., 2000; Ernst et al., 2000; Maini et al., 1990; Modi et al., 2002). Our longitudinal study in SIV monkeys is consistent with these findings. Taken together, our results and those of other investigators suggest that CBF may be valuable as a surrogate maker to assess the progression of $HIV^+$ patients or SIV-infected monkeys in the early period after viral infection.

### Cerebral atrophy estimation

Cerebral atrophy is often observed in $HIV^+$ patients (Dal Pan et al., 1992). To evaluate cerebral atrophy in this study, we used standardized planimetry to measure the ventricle-brain ratio (VBR) and the bifrontal ratio (BFR) and bicaudate ratio (BCR) for estimation of cerebral atrophy (Dal Pan et al., 1992; Hestad et al., 1993). No significant difference in VBR, BCR and BFR were observed after inoculation in comparison with the baseline in this study. Also, ANOVA test did not found any significant difference between any two different time points. However, the correlation analysis showed that the BFR changes were correlated significantly with CD4+ counts. Similar results of the BFR changes were reported in asymptomatic HIV-1-infected individuals (Hall et al., 1996). The BFR change suggests there may exist slight cerebral atrophy during the progression of the disease in this model, but more conclusive results should be obtained from a large cohort examination in the future.

### FA and MD in whole brain and ROI analysis

The whole brain FA was significantly reduced post inoculation (Fig 4). The result is consistent with findings in $HIV^+$ patients (Ragin et al., 2004). We also observed an apparent elevation of whole brain MD but this was only a non-significant trend, perhaps due to small sample size. It has also been reported that MD is less responsive than FA in HIV infection as

well (Ragin et al., 2004). The whole brain FA was decreased significantly in the 2$^{th}$ week post inoculation; this may imply that the whole brain FA changes are responding in the early stage. The significant correlation between the whole brain FA value and CD4+ counts indicate that DTI may be able to identify abnormal changes in the brain tissue in the earlier stage, and the whole brain FA is a sensitive marker for monitoring the advance in the progression of the disease (Pomara et al., 2001). In comparison, the MD values increased temporarily in the 4$^{th}$ week post inoculation, and then decreased gradually to the baseline level. No significant correlation between MD and T-cell counts was observed.

ROI analysis results before and after inoculation indicate that the FA in genu of corpus callosum declines in monkeys as it does in human patients with HIV-dementia (Thurnher et al., 2005) (Fig 5). Although there was a tendency for increases in MD in all the ROIs, there were no significant changes. This result is consistent with Thurnher et al's report (Thurnher et al., 2005), but different from Wu et al's (Wu et al., 2006), perhaps it is because of the small sample size in this study. In the longitudinal data, the FA value of splenium correlated significantly with CD4+ cell counts, and the correlation between the CD4:CD8 ratio and FA of frontal WM and genu are significant as well. These findings further suggest that DTI may be used to identify the abnormal changes in the earlier asymptomatic stage, and FA is a sensitive marker for monitoring the progression of the disease.

The FA and MD in the genu and splenium of corpus callosum have been used for monitoring disease progression in HIV$^+$ patients (Chang et al., 2008; Wu et al., 2006). In our longitudinal study, significantly decreased FA in the genu and whole brain were observed, and the reduction was significantly correlated with the CD4 count or CD4:CD8 ratio. This suggests that DTI is a potential tool to monitor the progression of HIV disease. In this study, it was found the significant changes in FA of whole brain (by post inoculation 2 weeks) appeared earlier than the significant changes in CBF (by post inoculation 4 weeks). FA may be more sensitive for detecting the progression of HIV-associated brain abnormities in earlier stage than other MRI modalities.

DTI provides information about the abnormality of white matter and is especially useful in studying the central nervous system (CNS) diseases associated with demyelination. Preliminary DTI studies in HIV$^+$ patients suggested that DTI is sensitive to subtle white matter abnormalities associated with HIV infection progression (Tucker et al., 2004). FA was observed decreased in whole brain, splenium and genu (Filippi et al., 2001; Thurnher et al., 2005; Wu et al., 2006) and frontal lobes (Pomara et al., 2001), while MD was increased in subcortical white matter (Filippi et al., 2001; Thurnher et al., 2005; Wu et al., 2006) in HIV$^+$ patient, and is significantly correlated with the severity of dementia (Filippi et al., 2001; Ragin et al., 2004; Ragin et al., 2005; Wu et al., 2006). Recent results (Chang et al., 2008; Chen et al., 2009; Pfefferbaum et al., 2009; Stebbins et al., 2007) further demonstrated that HIV infection contributes to progressive changes in FA and MD values, resulting from both direct loss of axonal integrity and complexity to the underlying axonal matrix (Chen et al., 2009; Pfefferbaum et al., 2009). Meanwhile, some studies found increased FA and decreased MD in several brain areas is suggesting the improvement of the disease (Schifitto et al., 2009). Overall, all these findings suggest that DTI is a sensitive marker for accessing the white matter abnormality in HIV$^+$ patients.

## Conclusions

Multi-parameter MRI measurement was performed longitudinally in a SIV infected macaque model of Neuro-AIDS. Although no significant changes were observed in cognitive behavior tests or in brain volume following viral inoculation, declines in CBF in caudate and parietal cortex regions and in FA in genu and whole brain were observed

significantly. Longitudinal changes in CBF of caudate, prefrontal cortex and parietal cortex and in FA of the whole brain, splenium were found to correlate significantly with the CD4+ T-cell depletion, while the changes in CBF of caudate and parietal cortex and FA in genu and frontal WM were observed correlated significantly with the CD4:CD8 ratio. These MRI findings suggest that CBF and FA may be sensitive image markers to track the progression of the infection and for characterizing the disease and treatment development of HIV infection. Further studies with a larger sample size are expected to reduce the variance of the CBF and FA values and blood cell counts as well.

## Acknowledgments

The authors are grateful to Drs Xiangyang Ma and Xiaoping Hu from Emory University for providing the segmented DTI sequence, Dr Jiongjiong Wang from University of Pennsylvania for providing the CASL sequence. All animal protocols were approved by the Institutional Animal Care and Use Committee of Emory University. The Yerkes National Primate Research Center is fully accredited by AAALAC. The project was supported in part by the base grant from the NIH/NCRR (P51 RR000165) and NIH grant MH067769 (to FJN).

## References

Ances BM, Roc AC, Wang J, Korczykowski M, Okawa J, Stern J, Kim J, Wolf R, Lawler K, Kolson DL, Detre JA. Caudate blood flow and volume are reduced in HIV+ neurocognitively impaired patients. Neurology. 2006; 66:862–866. [PubMed: 16567703]

Ances BM, Sisti D, Vaida F, Liang CL, Leontiev O, Perthen JE, Buxton RB, Benson D, Smith DM, Little SJ, Richman DD, Moore DJ, Ellis RJ. Resting cerebral blood flow: a potential biomarker of the effects of HIV in the brain. Neurology. 2009; 73:702–708. [PubMed: 19720977]

Arendt G. Imaging methods as a diagnostic tool in neuro-AIDS. A review. Bildgebung. 1995; 62:310–319. [PubMed: 8653003]

Bakshi R. Neuroimaging of HIV and AIDS related illnesses: a review. Front Biosci. 2004; 9:632–646. [PubMed: 14766397]

Burudi EM, Fox HS. Simian immunodeficiency virus model of HIV-induced central nervous system dysfunction. Adv Virus Res. 2001; 56:435–468. [PubMed: 11450309]

Chang L, Ernst T, Leonido-Yee M, Speck O. Perfusion MRI detects rCBF abnormalities in early stages of HIV-cognitive motor complex. Neurology. 2000; 54:389–396. [PubMed: 10668700]

Chang L, Wong V, Nakama H, Watters M, Ramones D, Miller EN, Cloak C, Ernst T. Greater than age-related changes in brain diffusion of HIV patients after 1 year. J Neuroimmune Pharmacol. 2008; 3:265–274. [PubMed: 18709469]

Chen Y, An H, Zhu H, Stone T, Smith JK, Hall C, Bullitt E, Shen D, Lin W. White matter abnormalities revealed by diffusion tensor imaging in non-demented and demented HIV+ patients. Neuroimage. 2009; 47:1154–1162. [PubMed: 19376246]

Comparet P, Darriet D, Jaffard R. [Demonstration of dissociation between frontal and temporal lesions in man on two versions of delayed non-matching recognition tests used in monkeys]. C R Acad Sci III. 1992; 314:515–518. [PubMed: 1521171]

Dal Pan GJ, McArthur JH, Aylward E, Selnes OA, Nance-Sproson TE, Kumar AJ, Mellits ED, McArthur JC. Patterns of cerebral atrophy in HIV-1-infected individuals: results of a quantitative MRI analysis. Neurology. 1992; 42:2125–2130. [PubMed: 1436522]

Descamps M, Hyare H, Zerizer I, H JAG. Neuroimaging of CNS involvement in HIV. J HIV Ther. 2008; 13:48–54. [PubMed: 19039295]

Eacott MJ, Gaffan D, Murray EA. Preserved recognition memory for small sets, and impaired stimulus identification for large sets, following rhinal cortex ablations in monkeys. Eur J Neurosci. 1994; 6:1466–1478. [PubMed: 8000570]

Ernst T, Itti E, Itti L, Chang L. Changes in cerebral metabolism are detected prior to perfusion changes in early HIV-CMC: A coregistered (1)H MRS and SPECT study. J Magn Reson Imaging. 2000; 12:859–865. [PubMed: 11105023]

Filippi CG, Ulug AM, Ryan E, Ferrando SJ, van Gorp W. Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain. AJNR Am J Neuroradiol. 2001; 22:277–283. [PubMed: 11156769]

Gandhi NS, Skolasky RL, Peters KB, Moxley RTt, Creighton J, Roosa HV, Selnes OA, McArthur J, Sacktor N. A comparison of performance-based measures of function in HIV-associated neurocognitive disorders. J Neurovirol. 2011

Gold LH, Fox HS, Henriksen SJ, Buchmeier MJ, Weed MR, Taffe MA, Huitron-Resendiz S, Horn TF, Bloom FE. Longitudinal analysis of behavioral, neurophysiological, viral and immunological effects of SIV infection in rhesus monkeys. J Med Primatol. 1998; 27:104–112. [PubMed: 9747951]

Gongvatana A, Schweinsburg BC, Taylor MJ, Theilmann RJ, Letendre SL, Alhassoon OM, Jacobus J, Woods SP, Jernigan TL, Ellis RJ, Frank LR, Grant I. White matter tract injury and cognitive impairment in human immunodeficiency virus-infected individuals. J Neurovirol. 2009; 15:187–195. [PubMed: 19306228]

Gorman AA, Foley JM, Ettenhofer ML, Hinkin CH, van Gorp WG. Functional consequences of HIV-associated neuropsychological impairment. Neuropsychol Rev. 2009; 19:186–203. [PubMed: 19472057]

Grant I, Heaton RK, Atkinson JH. Neurocognitive disorders in HIV-1 infection. HNRC Group. HIV Neurobehavioral Research Center. Curr Top Microbiol Immunol. 1995; 202:11–32. [PubMed: 7587358]

Grassi MP, Perin C, Borella M, Mangoni A. Assessment of cognitive function in asymptomatic HIV-positive subjects. Eur Neurol. 1999; 42:225–229. [PubMed: 10567820]

Hall M, Whaley R, Robertson K, Hamby S, Wilkins J, Hall C. The correlation between neuropsychological and neuroanatomic changes over time in asymptomatic and symptomatic HIV-1-infected individuals. Neurology. 1996; 46:1697–1702. [PubMed: 8649573]

Herndon JG, Moss MB, Rosene DL, Killiany RJ. Patterns of cognitive decline in aged rhesus monkeys. Behav Brain Res. 1997; 87:25–34. [PubMed: 9331471]

Herscovitch P, Raichle ME. What is the correct value for the brain--blood partition coefficient for water? J Cereb Blood Flow Metab. 1985; 5:65–69. [PubMed: 3871783]

Hestad K, McArthur JH, Dal Pan GJ, Selnes OA, Nance-Sproson TE, Aylward E, Mathews VP, McArthur JC. Regional brain atrophy in HIV-1 infection: association with specific neuropsychological test performance. Acta Neurol Scand. 1993; 88:112–118. [PubMed: 8213054]

Lentz MR, Kim WK, Lee V, Bazner S, Halpern EF, Venna N, Williams K, Rosenberg ES, Gonzalez RG. Changes in MRS neuronal markers and T cell phenotypes observed during early HIV infection. Neurology. 2009; 72:1465–1472. [PubMed: 19398702]

Letendre SL, Ellis RJ, Ances BM, McCutchan JA. Neurologic complications of HIV disease and their treatment. Top HIV Med. 2010; 18:45–55. [PubMed: 20516524]

Lu H, Clingman C, Golay X, van Zijl PC. Determining the longitudinal relaxation time (T1) of blood at 3.0 Tesla. Magn Reson Med. 2004; 52:679–682. [PubMed: 15334591]

Maini CL, Pigorini F, Pau FM, Volpini V, Galgani S, Rosci MA, Narciso P. Cortical cerebral blood flow in HIV-1-related dementia complex. Nucl Med Commun. 1990; 11:639–648. [PubMed: 2234697]

Martin EM, Pitrak DL, Pursell KJ, Mullane KM, Novak RM. Delayed recognition memory span in HIV-1 infection. J Int Neuropsychol Soc. 1995; 1:575–580. [PubMed: 9375245]

Modi G, Modi M, Martinus I, Vangu M. New onset seizures in HIV-infected patients without intracranial mass lesions or meningitis--a clinical, radiological and SPECT scan study. J Neurol Sci. 2002; 202:29–34. [PubMed: 12220689]

Muller-Oehring EM, Schulte T, Rosenbloom MJ, Pfefferbaum A, Sullivan EV. Callosal degradation in HIV-1 infection predicts hierarchical perception: a DTI study. Neuropsychologia. 2010; 48:1133–1143. [PubMed: 20018201]

Murray EA, Rausch DM, Lendvay J, Sharer LR, Eiden LE. Cognitive and motor impairments associated with SIV infection in rhesus monkeys. Science. 1992; 255:1246–1249. [PubMed: 1546323]

Novembre FJ, De Rosayro J, O'Neil SP, Anderson DC, Klumpp SA, McClure HM. Isolation and characterization of a neuropathogenic simian immunodeficiency virus derived from a sooty mangabey. J Virol. 1998; 72:8841–8851. [PubMed: 9765429]

Novembre FJ, Saucier MM, Hirsch VM, Johnson PR, McClure HM. Viral genetic determinants in SIVsmmPBj pathogenesis. J Med Primatol. 1994; 23:136–145. [PubMed: 7966228]

O'Neil SP, Suwyn C, Anderson DC, Niedziela G, Bradley J, Novembre FJ, Herndon JG, McClure HM. Correlation of acute humoral response with brain virus burden and survival time in pig-tailed macaques infected with the neurovirulent simian immunodeficiency virus SIVsmmFGb. Am J Pathol. 2004; 164:1157–1172. [PubMed: 15039205]

Pfefferbaum A, Rosenbloom MJ, Adalsteinsson E, Sullivan EV. Diffusion tensor imaging with quantitative fibre tracking in HIV infection and alcoholism comorbidity: synergistic white matter damage. Brain. 2007; 130:48–64. [PubMed: 16959813]

Pfefferbaum A, Rosenbloom MJ, Rohlfing T, Kemper CA, Deresinski S, Sullivan EV. Frontostriatal fiber bundle compromise in HIV infection without dementia. AIDS. 2009; 23:1977–1985. [PubMed: 19730350]

Pomara N, Crandall DT, Choi SJ, Johnson G, Lim KO. White matter abnormalities in HIV-1 infection: a diffusion tensor imaging study. Psychiatry Res. 2001; 106:15–24. [PubMed: 11231096]

Ragin AB, Storey P, Cohen BA, Epstein LG, Edelman RR. Whole brain diffusion tensor imaging in HIV-associated cognitive impairment. AJNR Am J Neuroradiol. 2004; 25:195–200. [PubMed: 14970017]

Ragin AB, Wu Y, Storey P, Cohen BA, Edelman RR, Epstein LG. Diffusion tensor imaging of subcortical brain injury in patients infected with human immunodeficiency virus. J Neurovirol. 2005; 11:292–298. [PubMed: 16036809]

Sahakian BJ, Elliott R, Low N, Mehta M, Clark RT, Pozniak AL. Neuropsychological deficits in tests of executive function in asymptomatic and symptomatic HIV-1 seropositive men. Psychol Med. 1995; 25:1233–1246. [PubMed: 8637953]

Schifitto G, Zhong J, Gill D, Peterson DR, Gaugh MD, Zhu T, Tivarus M, Cruttenden K, Maggirwar SB, Gendelman HE, Dewhurst S, Gelbard HA. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. J Neurovirol. 2009; 15:176–186. [PubMed: 19306230]

Stebbins GT, Smith CA, Bartt RE, Kessler HA, Adeyemi OM, Martin E, Cox JL, Bammer R, Moseley ME. HIV-associated alterations in normal-appearing white matter: a voxel-wise diffusion tensor imaging study. J Acquir Immune Defic Syndr. 2007; 46:564–573. [PubMed: 18193498]

Strupp JP. Stimulate: a GUI based fMRI analysis software package. Neuroimage. 1996; 3:S607.

Thurnher MM, Castillo M, Stadler A, Rieger A, Schmid B, Sundgren PC. Diffusion-tensor MR imaging of the brain in human immunodeficiency virus-positive patients. AJNR Am J Neuroradiol. 2005; 26:2275–2281. [PubMed: 16219833]

Thurnher MM, Donovan Post MJ. Neuroimaging in the brain in HIV-1-infected patients. Neuroimaging Clin N Am. 2008; 18:93–117. viii. [PubMed: 18319157]

Tucker KA, Robertson KR, Lin W, Smith JK, An H, Chen Y, Aylward SR, Hall CD. Neuroimaging in human immunodeficiency virus infection. J Neuroimmunol. 2004; 157:153–162. [PubMed: 15579293]

Wang J, Zhang Y, Wolf RL, Roc AC, Alsop DC, Detre JA. Amplitude-modulated continuous arterial spin-labeling 3.0-T perfusion MR imaging with a single coil: feasibility study. Radiology. 2005; 235:218–228. [PubMed: 15716390]

Williams R, Bokhari S, Silverstein P, Pinson D, Kumar A, Buch S. Nonhuman primate models of NeuroAIDS. J Neurovirol. 2008; 14:292–300. [PubMed: 18780230]

Woods SP, Moore DJ, Weber E, Grant I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. Neuropsychol Rev. 2009; 19:152–168. [PubMed: 19462243]

Wu Y, Storey P, Cohen BA, Epstein LG, Edelman RR, Ragin AB. Diffusion alterations in corpus callosum of patients with HIV. AJNR Am J Neuroradiol. 2006; 27:656–660. [PubMed: 16552012]

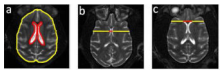


**Fig. 1.**

Brain atrophy index estimation. a) The ventricle-brain ratio (VBR): the area of lateral ventricle (marked by red line) divided by the whole brain area in the same slice (marked by yellow line); b) The bicaudate ratio (BCR): the minimum inter-caudate distance (marked by red line) divided by the correspondent brain width in the same slice (marked by yellow line) ; c) The bifrontal ratio (BFR): the distance between the lateral tips of the frontal horns (marked by red line) divided by the correspondent brain width in the same slice (marked by yellow line).

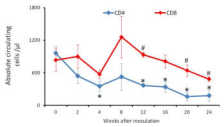


**Fig. 2.**

CD4+ and CD8+ T-cell count changes in macaques after SIV inoculation. Error bars represent standard deviation error. *, # : P <0.05 compared with the baseline (before SIV inoculation) and 16$^{th}$ week, respectively.

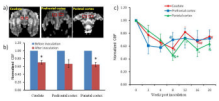

**Fig. 3.**

Normalized Cerebral Blood Flow (CBF) changes in the caudate, frontal cortex and inferior medial parietal lobe after SIV inoculation. a) CBF maps acquired with the Continuous Arterial Spin Labeling (CASL) technique. Regions of Interest (ROIs) are marked by red lines. b) CBF changes in selected brain regions before and after SIV inoculation. c) Longitudinal CBF changes after SIV inoculation. Error bars represent standard deviation error. *, # : P <0.05 compared with baseline and 12$^{th}$ week, respectively, in caudate; @: P <0.05 compared with baseline in prefrontal cortex; &,+: P <0.05 compared with the baseline and 2$^{nd}$ week, respectively, in parietal cortex.

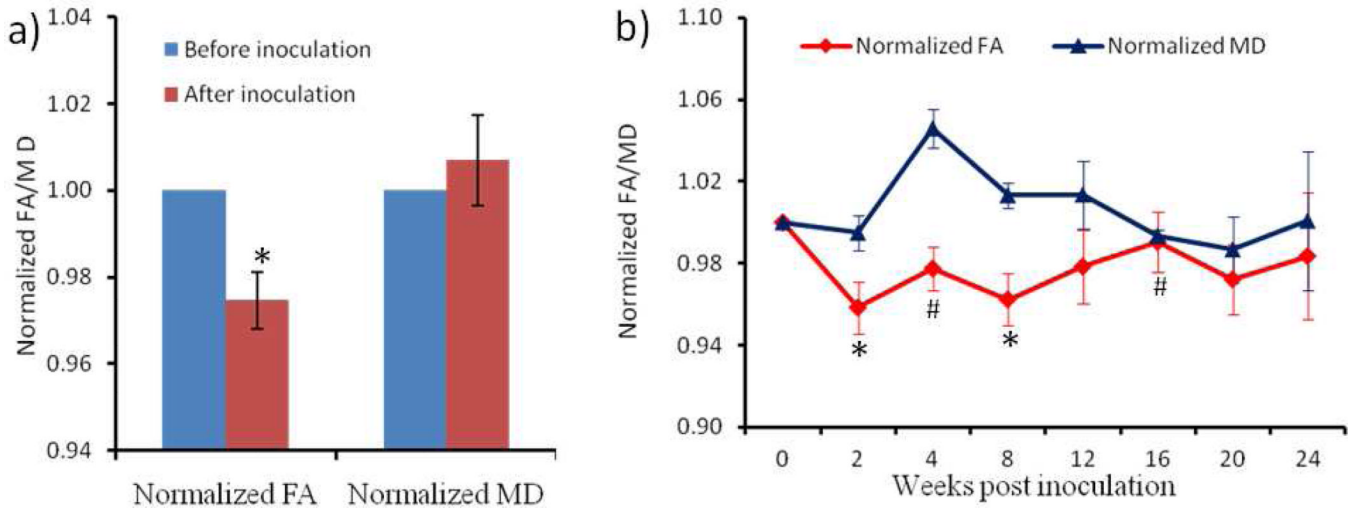


**Fig. 4.**

Normalized Fractional Anisotropy (FA) and Mean Diffusivity (MD) changes of whole brain after SIV inoculation. a) The whole brain FA and MD difference before and after SIV inoculation. b) Longitudinal changes of whole brain FA and MD after SIV inoculation. Error bars represent standard deviation error. *, # : $P<0.05$ compared with the baseline and 2nd week.

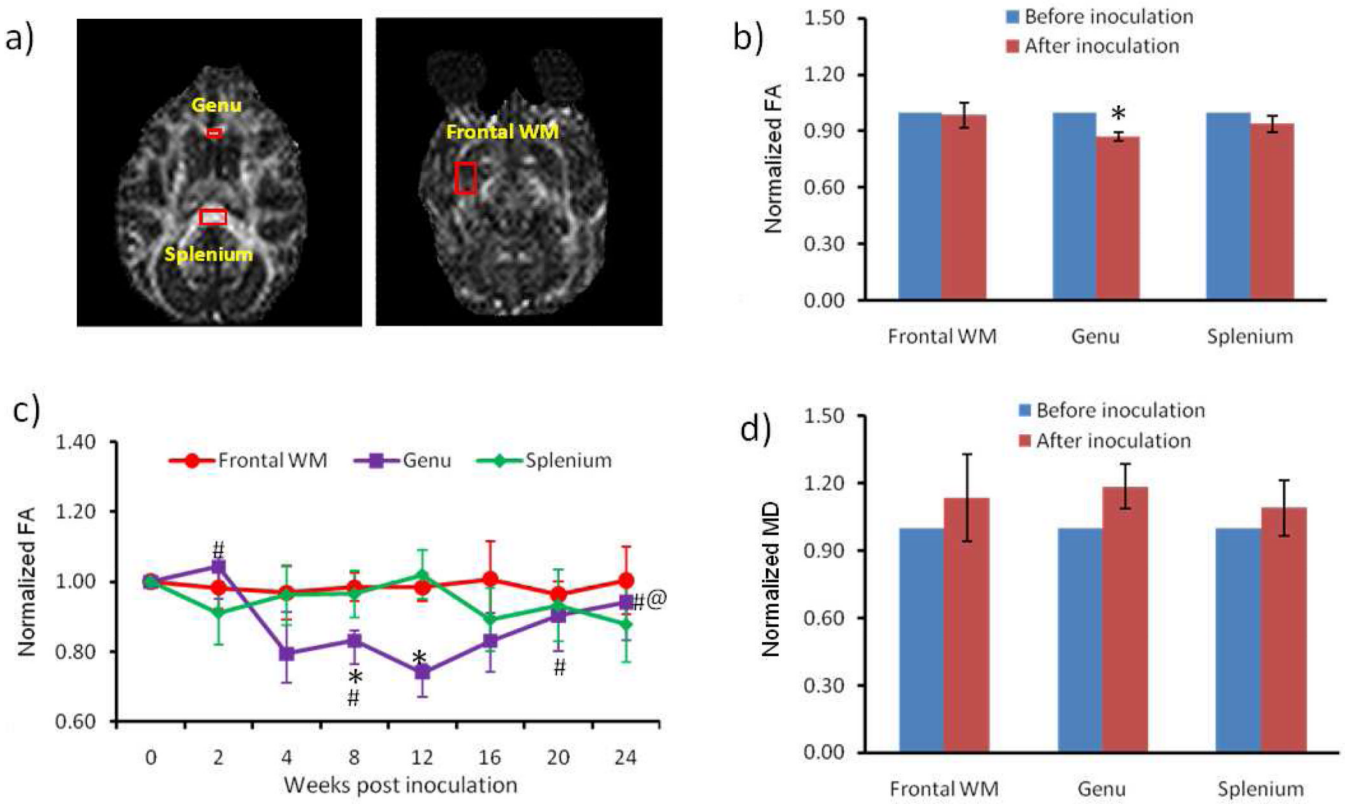


**Fig. 5.**

Normalized Fractional Anisotropy (FA) and Mean Diffusivity (MD) changes in the selected ROIs (genu, splenium, and frontal white matter) after SIV inoculation. a) The ROIs are illustrated on the FA map of a monkey brain. b) Mean FA differences before and after SIV inoculation. c) Longitudinal FA changes in genu, splenium, and frontal white matter. d) Mean MD differences before and after SIV inoculation. Error bars represent standard deviation error. *, @, #: $P < 0.05$ compared with the baseline, $8^{th}$ week and $12^{th}$ week, respectively, in genu.

## Table 1

Correlations between T-cell counts (CD4 and CD4:CD8 ratio) and brain atrophy indexes (VBR, BCR and BFR).

| T-Cell count | VBR | BCR | BFR |
|---|---|---|---|
| CD4 | 0.29 | 0.25 | −0.50* |
| CD4/CD8 | 0.27 | 0.15 | −0.01 |

*$P<0.05$

**Table 2**

Correlations between T-cell counts (CD4 and CD4:CD8 ratio) and CBF in different ROIs.

| T-Cell count | Caudate | Prefrontal cortex | Parietal cortex |
|---|---|---|---|
| CD4 | 0.48* | 0.48* | 0.55* |
| CD4/CD8 | 0.48* | 0.33 | 0.71* |

*$P<0.05$

## Table 3

Correlations of CD4 counts and CD4:CD8 ratio with FA in whole brain and selected ROIs.

| Cell count | Whole brain | Genu | Splenium | Frontal WM |
|---|---|---|---|---|
| CD4 | 0.49* | 0.35 | 0.47* | 0.36 |
| CD4/CD8 | 0.28 | 0.41* | 0.36 | 0.44* |

* $P<0.05$